CULTURAL VARIATION IN *MYCOSPHAERELLA FRAGARIAE* (TUL.) LINDAU.

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ABSTRACT. – A detailed study on the cultural characteristics of 6 isolates of *M. fragariae* (Tul.) Lindau collected in the Auckland region from the strawberry variety Tioga was carried out. All isolates could be distinguished when grown on PDA at 25°C in darkness for 21 days, using the following characteristics : colour of mycelium, texture of mycelium, spore production, growth rate, and pigment exudation both onto the mycelium and into the growth medium.

Cultural variation within isolates was also studied. *M. fragariae* was found to be extremely variable in culture, variation of juvenile colonies from parents in appearance ranging from 0-40 % between replicates. All combinations of mycelial colour (red, grey, pink, white), dye production (profuse, slight, none) and ability to colour the growth medium were observed.

RÉSUMÉ. – Les caractéristiques culturales de 6 isolats de *Mycosphaerella fragariae* (Tul.) Lindau sont étudiées. Ils ont été prélevés sur la variété de fraisier Tioga dans la région d'Auckland. Tous les isolats sont distincts après culture sur PDA pendant 21 jours à 25°C dans l'obscurité, sur la base des caractères suivants : couleur et texture du mycélium, production de spores, croissance, exudations pigmentées en surface et dans le milieu.

La variation des isolats en culture a été étudiée. *M. fragariae* présente un taux de variation très élevé; il est compris entre 0 et 40 % suivant les repiquages, pour les différences entre colonies juvéniles et parentales. Toutes les combinaisons de couleurs (rouge, rose, gris, blanc), de production de pigment (profuse, faible, nulle), et de capacité à colorer le milieu de culture ont été observées.

KEY WORDS : Strawberry, *Mycosphaerella fragariae, Fragaria,* leaf-spot, cultural variation.

INTRODUCTION

Strawberry Leaf Spot or Frog Eye disease caused by *Mycosphaerella fragariae* (Tul.) Lindau, is one of the most common fungus diseases affecting strawberries worldwide (PLAKIDAS, 1964; ALEXANDER, 1982). During a study of the pathogenicity of various *M. fragariae* isolates on a range of strawberry varieties, extreme cultural variation was observed both between and within isolates of

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the fungus. The existence of variants of *M. fragariae* distinguishable in culture by macroscopic and microscopic characteristics has been established previously (PALCHEFSKY & ALLISON, 1950; BOLTON, 1958, 1962; NEMEC, 1969). PALCHEFSKY & ALLISON (1950) claimed to have isolated two distinct cultural types of *M. fragariae* from different leaflets of a single leaf. BOLTON (1962) however, recognised forty-nine unique cultural variants of *M. fragariae* and proposed that it was the variety of strawberry from which the isolate was derived that determined the cultural characteristics of that isolate.

This paper describes the isolation of *M. fragariae* from different sources in the Auckland region of New Zealand, and evaluates the cultural characteristics of each of the isolates.

MATERIALS AND METHODS

Isolation of *M. fragariae*.

Leaves of the variety Tioga showing mature Leaf Spot symptoms were collected from four strawberry plantations in the north Auckland area. The detached leaves were washed in 3 % sodium hypochlorite for 4 min and subsequently rinsed in sterile distilled water (SDW). Leaf spots were then dissected out and cut in half. The halves were placed vertically onto Potato Dextrose Agar (PDA) and incubated at 25°C in the dark. White tufts of conidiophores could be seen growing from the cut edges of the leaf spots above the agar after 2 days. Conidia were picked off under a dissecting microscope using a sterile needle, placed onto fresh PDA plates and incubated at 25°C in darkness. In this way four isolates were obtained and were designated 101, 102, 103 and 104 respectively. Isolate 103 which often failed to grow on PDA, was maintained on a stock culture of autoclaved Tioga leaves which were placed on the top of a thin layer of PDA in petri dishes, while the remaining isolates were maintained on PDA, at 25°C in darkness.

The two isolates designated 8028 and 6M were supplied by Dr W. Hartill, Plant Diseases Division, DSIR, Auckland.

Growth of *M. fragariae* colonies.

Single colonies for observation and comparison were obtained either by subculturing 2 mm diameter mycelial discs, or by plating out single spores which had been germinated in a solidified 1 % glucose/1.8 % agar mix containing the spore suspension. Single spores were picked out from these plates after 4 days with the aid of a dissecting microscope (BOLTON, 1962). All observations were made on 21 day old colonies grown on PDA in darkness at 25°C.

Cultural characteristics.

Observations on colour of mycelium, surface texture, and pigment exudation onto the surface of the mycelium were made for colonies grown from mycelial discs.

The exudation of pigment into the agar medium was measured for colonies grown from single spores using the following method. Four 1 cm diameter discs of agar were cut from around the growing edge of 5 replicate colonies per isolate. Each disc was placed in a test tube containing 3 ml of SDW and left overnight at 3°C to allow the pigment to diffuse from the agar. The solution was filtered and absorbance recorded using a Perkin spectrophotometer at 480 nm and 360 nm against appropriate controls.

Colony growth was assessed for each isolate by measuring the diameter of 12 replicate colonies.

Conidial production was determined by detaching the colonies from the agar surface using forceps and placing each in a test tube containing 2 ml of SDW. The tubes were agitated on a vortex mixer until a consistent spore count was recorded using a haemocytometer.

Variation of juvenile colonies from the parent was assessed by comparing the colour, texture, and level of pigment production of a number of single spore colonies with that of the parent colony.

Variation in colony characteristics was also observed over a period of 18 months. Variants obtained were subcultured and held in a stock collection for comparison with parent colonies.

RESULTS

Individual isolates were able to be distinguished on the basis of the characteristics shown in Table 1. Isolate 6M was unique in both mycelial colour and texture (Fig. 1A), no other isolate having such a pale colour and matted appearance. Isolate 101 was also easily distinguished by the formation of large pigment droplets on the mycelial (Fig. 1B). Isolate 8028 was unique in its formation of a darker band around the edge of the colony (Fig. 1C), while isolates 102, 103, and 104 were all similar in appearance (Fig. 1D).

Isolates 8028 and 6M both had high growth rates but were poor sporulators. Greatest sporulation was observed for isolate 103, although problems were encountered in culturing this isolate on PDA.

The ability to colour the medium varied considerably between isolates, with greatest pigment exudation by isolates 101 and 102.

Attempts to quantify the degree of variation in visual characteristics of juvenile colonies from their parents met with little success. The percentage of colonies differing from parent colonies ranged from 0-40 % between replicates carried out over time. It was possible, however, to distinguish some isolates by the sectors they commonly produced (a sector being a spontaneous change in either the colour or texture of the mycelium). Isolate 101 produced white sectors almost exclusively while isolate 8028 produced sectors with a matted texture which were pale in colour.

The rate at which variant sectors appeared also varied over time. Isolate 8028 produced four variants within the first six months of observation. It then remai-

Characteristics	Isolate 8028	6M	101	102	103	104
Colour of mycelium	pink with red border	pale pink	red with grey border	red with grey border	grey til 0.5mm then red	red with grey border
Texture of mycelium	felt[I]	matted[II]	felt	felt	felt	felt
Pigmented droplets (no. and diameter in mm)	few <=1	none	many 2 - 3	few <=1	few 2 - 4	few <=1
Colony diameter (mm)	10.3 (1.4)	7.6 (1.2)	6.8 (0.6)	7.6 (1.2)	7.6 (1.0)	6.8 (1.4)
Conidia production ($x10^5$)	1.20 (0.14)	1.20 (0.19)	1.20 (0.43)	2.00 (0.16)	5.00 (0.70)	2.33 (0.22)
Pigment Exudation (A480nm - 360nm)	0.70 (0.01)	1.05 (0.32)	1.77 (0.49)	1.73 (0.56)	[III]	0.65 (0.22)
Common sectors observed	matted	deep red or matted	white	none	matted fur or white felt	[illegible]

I A long stranded mycelial colony with the aerial mycelium matted together like wet fur.
II Mycelium short and dense (felt-like).
III Colonies failed to grow on PDA.

Table 1 – Characteristics of *M. fragariae* isolates grown on PDA at 25°C in darkness for 21 days. Numerical values shown are means (with SD).

Tableau 1 – Caractères des isolats de *M. fragariae* après culture pendant 21 jours sur PDA à 25°C dans l'obscurité.

ned stable for four months before all remaining colonies of the parent type changed to a pale pink colour. Isolate 6M in contrast, remained stable for six months and then produced three variants in the space of three weeks. From the six isolates held in culture over 18 months, variants exhibiting almost all combinations of mycelium colour (red, white, grey, pink), pigment production (profuse, slight, none), and ability to colour the growth medium were observed. Variants obtained included a white nonsporulating colony with no aerial mycelium derived from 6M, and a variant identical in appearance to 6M but having an extremely fast growth rate.

Variants differed in stability also, some remaining stable in culture for many months, while others quickly reverted to their parent form or to yet another variation. The stability of any particular variant was unrelated to its cultural characteristics.

DISCUSSION

All six isolates of *M. fragariae* studied could be distinguished using the seven cultural characteristics tested i.e. colour and texture of the mycelium, spore production, growth rate, pigment exudation (both onto the mycelium and into

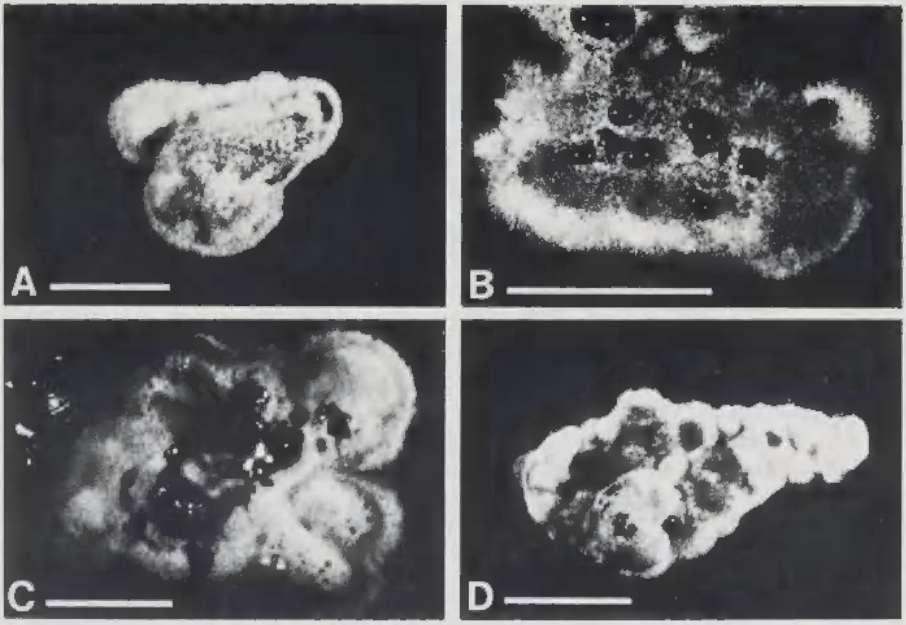

Figure 1 — Three week old colonies of *Mycosphaerella fragariae* grown on PDA at 25°C in darkness. Isolates (A) 6M, (B) 101, (C) 8028, (D) 103. Scale bar : 1 cm.

Figure 1 — Colonies de 3 semaines de *Mycosphaerella fragariae* cultivées sur PDA à 25°C dans l'obscurité. Isolats (A) 6M, (B) 101, (C) 8028, (D) 103. Échelle : 1 cm.

the medium), and sectors produced. Many of the variations seen in these characteristics are similar to those previously reported in the literature for *M. fragariae* (PALCHEFSKY & ALLISON, 1950; BOLTON, 1958), indicating that the diversity of this fungus in New Zealand is as extensive as that seen elsewhere. Spontaneous changes in macroscopic characteristics also occurred readily in culture, some isolates exhibiting a greater frequency of change than others. Variation usually occurred by sector production and occasionally by whole colony changes soon after subculturing.

Such extreme variation in culture is not limited to *M. fragariae*. *Mycosphaerella nubilosa* (Cooke) Hansf. (GANAPATHI, 1979), *Botrytis cinerea* Pers. ex Fr. (JARVIS, 1977), and *Penicillium* species (BRIDGE & al., 1986) have also been noted for the heteromorphic nature of their colonies. The mechanism for such changes in *M. fragariae* has not been determined, though in *Penicillium* it has been suggested to be parasexual recombination (BURNETT, 1976).

The variation shown in the cultural characteristics both between and within isolates of *M. fragariae* raises several points. Firstly, it brings into doubt claims that the isolate type is determined by the variety of strawberry from which it is obtained (BOLTON, 1962), as all six of the isolates used in this study were

from the variety Tioga. In contrast however, it has also been shown that variation in cultural type can be introduced early in the subculturing process, perhaps explaining the isolation of two distinct cultural types from one leaf by PALCHEFSKY & ALLISON (1950). Secondly, it has previously been shown that variations within isolates can have an effect on morphological and physiological characteristics, and on secondary metabolite production (SANSOME, 1949; SCHAREN & KRUPINSKY, 1970). It is possible that variation in culture is therefore occurring with those characteristics that affect survivorship and adaptability in the field such as growth and sporulation rates. Though BOLTON (1962) showed that the cultural characteristics of *M. fragariae* isolates did not correlate with pathogenicity when using freshly obtained isolates, the question of the reliability of pathogenicity tests using isolates held in culture for some time remains. Under conditions of repeated culture and preservation many changes may occur in the characteristics of an isolate. It may therefore differ significantly from the isolate taken from the field by the time pathogenicity trials or other observations are carried out.

Finally, the question of how isolate variation occurring in the field affects disease development is raised. Is it this particular characteristic of *M. fragariae* which has allowed it to remain a serious pathogen on a wide range of strawberry cultivars worldwide, despite the constant development of new varieties of strawberry and new fungicides for its control ? If so, then this particular characteristic of *M. fragariae* warrants further investigation if a successful disease management strategy is to be maintained.

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